

Characteristics of Bacteria Associated with the Gastropod *Physa integra* (Haldeman)

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INTRODUCTION

MOST STUDIES of bacterial associations with mollusks have been done in the area of culturing embryos (WILBUR & YOUNG, 1964) and increasing interest of bacterial relationships is being shown in associations dealing with gastropod pathology (BAYNE & KIME, 1970).

This report describes bacteria associated with *Physa integra* (HALDEMAN, 1841). In this report the word "associated" is used broadly in the sense of found together or on *Physa*.

COLLECTION OF THE BACTERIAL SPECIMENS

Twenty adult specimens of *Physa* were collected from a drainage ditch leading into the Sawkill, a tributary of the

Hudson River in the vicinity of Red Hook, New York.

The animals were then immediately brought to the laboratory where the apices of the shells were used to streak culture plates of nutrient agar, 0.1% peptone, and peptonized milk agar which were then incubated at 20° C. In addition, separate scrapings were taken from the shell apices, whorl sutures and the inner surface of the aperture of the shell and cultured with the above media. These were then incubated at 20° C and subsequent isolations were made within 36 hours at 20° C.

The habitat from which the animals were collected was characterized by a water temperature of 25° C, a silt bottom, and considerable decaying plant matter. Solid surfaces of the collection area consisted to a high degree of the shells of *Physa*, other invertebrates, and small rocks.

Table 1

Physiological characteristics of bacteria isolated from *Physa*

Bacterial genus	A	B	C	D	E	F	G	H	I	J	K	L	M
<i>Achromobacter</i>	—	—	—	—	—	—	—	—	+	—	—	—	+
<i>Aerobacter</i>	a	—	—	—	—	+	+	+	+	—	—	+	—
<i>Aeromonas</i>	a/g	—	—	+	—	+	+	—	+	a	—	+	+
<i>Alcaligenes</i>	a	—	—	—	—	+	+	+	—	—	—	—	—
<i>Bacillus</i>	a	a	—	—	—	+	+	+	+	a	—	+	+
<i>Flavobacterium</i>	—	—	—	—	—	—	—	+	—	—	—	—	—
<i>Salmonella</i> (?)	a/g	—	—	+	+	—	+	—	+	a	—	+	+

Legend:

A—glucose; B—lactose; C—Indole production; D—H₂S production; E—methyl red test; F—acetylmethylcarbinol production; G—citrate utilization; H—gelatin liquefaction; I—nitrate utilization; J—reduction of litmus milk; K—urease production; L—Hugh-Leifson glucose aerobic; M—Hugh-Leifson glucose anaerobic; a—acid; g—gas. Blank spaces indicate no test performed.

Table 2

Differential generic characteristics of bacteria associated with *Physa*

Bacterial genus	Gram stain	Spores	Pigment	Flagella	Motility	Hugh-Leifson glucose
<i>Achromobacter</i>	—	—	—	—	—	fermentative
<i>Aerobacter</i>	—	—	+	—	—	oxidative
<i>Aeromonas</i>	—	—	+	polar	+	oxidative & fermentative
<i>Alcaligenes</i>	—	—	—	—	—	negative
<i>Bacillus</i>	+	+	+	peritrichous	+	slightly oxidative & fermentative
<i>Flavobacterium</i>	—	—	—	—	—	fermentative
<i>Microcylus</i>	—	—	—	—	—	fermentative
<i>Salmonella</i> (?)	—	—	—	—	—	oxidative & fermentative
<i>Spirillum</i>	—	—	—	bipolar	+	no growth
<i>Vibrio</i>	—	—	—	polar	+	

Blank spaces indicate characteristic was not investigated

GENERIC DETERMINATION

The general procedures of generic determination follow that of JOHNSON, SCHWENT, & PRESS (1968) with appropriate modifications to the freshwater environment. All cultures were Gram stained, examined microscopically, and a series of physiological tests were made (Table 1) by standard methods (PELCAZAR, 1957) using sterile filtered water from the natural habitat of the animals. Glucose metabolism was determined by the method of HUGH & LEIFSEN (1953). Cultures were tested for H₂S production by the method of SKERMAN (1959). Table 2 shows the key characteristics used in differentiating the bacterial genera.

RESULTS AND DISCUSSION

Achromobacter, *Alcaligenes*, *Flavobacterium*, and *Salmonella* were isolated on nutrient agar, while *Aerobacter*, *Aeromonas*, and *Bacillus* were isolated on peptonized milk agar. The majority of the bacterial isolates were gram negative rods with the exception of *Bacillus*. No antigenic analysis was done with the *Salmonella* suspect. Though *Microcylus* was not isolated it was observed in numerous samples taken from the apices. *Vibrio*-like and *Spirillum*-like species were not isolated but were observed in all samples. The cells of *Spirillum* most often illustrated extremely long screw-like turns.

In general, there did not seem to be an association between an isolated bacterial genus and a conchological area

from which samples were taken. Samples from the apices yielded the most numerous isolates. Isolates were not obtained from the inner part of the aperture.

The association of bacteria with the shell of these gastropods is not surprising in that bacteria readily develop in small bodies of water where the area of solid surface is high compared with the volume of water (WALNE, 1958; GUILLARD, 1959). In addition, Bergey's Manual of Determinative Bacteriology (1957) lists the bacteria of this study as either found in water or commonly found in nature which may imply that the association of these bacteria with *Physa* is not unusual. Whether there is an interspecific relationship between *Physa* and a bacterial genus or group of bacterial genera was not determined. It appears that in the natural habitat the bacterial genera of this study may be associated with *Physa* in the neutralistic sense of ODUM (1971).

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